

Diffusion Potential Studies: Their Application
to the Systems Hydrochloric Acid-Sodium
Chloride, Hydrochloric Acid-Gelatin
and a New Method for the De-
termination of the Equiva-
lent Weight of Gelatin

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE
UNIVERSITY OF MICHIGAN IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

By
Egbert King Bacon
June, 1926

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN]

DIFFUSION-POTENTIAL MEASUREMENTS APPLIED TO HYDROCHLORIC ACID-GELATIN SYSTEMS. I. THE EQUIVALENT WEIGHT OF GELATIN¹

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In this article an apparatus is described which permits the approximate measurement of a static liquid-junction potential between hydrochloric acid of two concentrations and of more complex systems of hydrochloric acid-sodium chloride. The measurements are also extended to systems of hydrochloric acid-gelatin. The striking similarity between these systems and those of hydrochloric acid-sodium chloride favors the establishment of gelatin-acid solutions as true solutions. The stoichiometric character of the combination between gelatin and acid is demonstrated and the equivalent weight of gelatin determined.

If two different electrolytic solutions are placed in contact, there is developed at the junction a potential difference which is called a diffusion potential. This potential is caused by the unequal migration velocities of the ions in the two solutions, that is, in simple electrolytes or mixtures of electrolytes the potentials are determined by the ionic activities and the ionic mobilities.

In a boundary such as



the potential is zero since identical solutions are on each side of the contact layer. If, however, sodium hydroxide replaces part of the hydrochloric acid in one of the solutions in such a manner that the mixture always has a constant concentration of 0.10 *N*, a potential develops which becomes increasingly greater as the solution ultimately approaches 0.10 *N* sodium chloride, since the hydrogen ion is being replaced by the slower-moving sodium ion.

If in an identical arrangement as the above gelatin is substituted for the sodium hydroxide, the resulting potentials should give some information as to the physical state of the resulting mixtures, that is, whether as Loeb and others have maintained, gelatin reacts stoichiometrically with hydrochloric acid, forming highly ionized gelatin chloride,² or whether an adsorption complex of some sort is formed,³ or whether the type of

¹ The material presented in this article is constructed from a portion of the thesis submitted to the Graduate School of the University of Michigan by Egbert K. Bacon in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² (a) Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Co., New York, 1922. (b) Hitchcock, *J. Gen. Physiol.*, 5, 383 (1923). (c) Procter, *J. Chem. Soc.*, 105, 313 (1914).

³ (a) Shukov and Shchukarev, *J. Phys. Chem.*, 29, 285 (1925). (b) Alexander, "Glue and Gelatin," Chemical Catalog Co., New York, 1923. (c) de Izaguirre, *Kolloid-Z.*, 32, 47 (1923). (d) Bracewell, *THIS JOURNAL*, 41, 1511 (1919).

combination might be due partly to purely chemical forces and partly to forces of adsorption, as Hoffman and Gortner suggest.⁴

In order to carry out such measurements it was necessary to devise an apparatus that would permit the measurement of such potentials between solutions of high viscosity. Its reliability was first demonstrated with simpler electrolytic solutions.

Hydrochloric Acid Cells

The apparatus used in the investigation is illustrated by Fig. 1. A and B are two glass vessels filled with solutions M and M'. CGD is a three-way stopcock of 9 mm. bore. The two siphon arms are indicated.

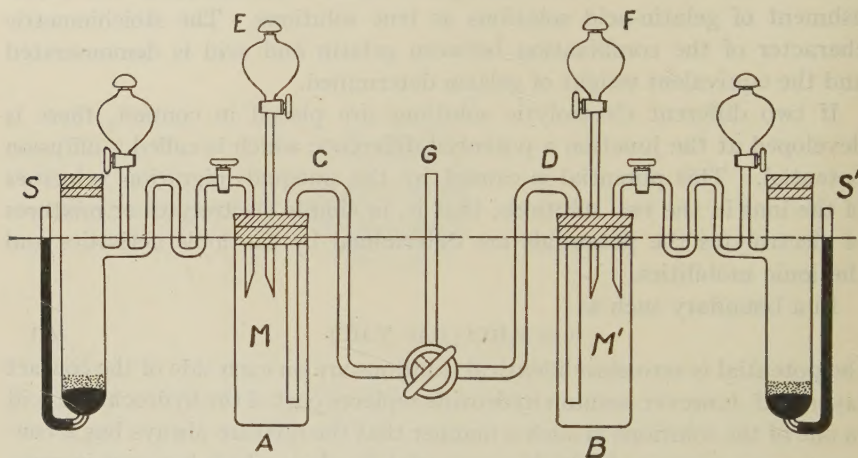
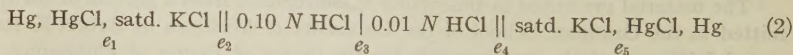


Fig. 1.—Apparatus with saturated potassium chloride electrodes for the measurement of diffusion potentials.

E and F are dropping funnels and S and S' saturated potassium chloride electrodes. The liquid junction was made in the stopcock between the two siphon arms by proper manipulation of the stopcock and the application of suction through G.

Potential measurements were first made with a simple boundary which is illustrated by the following cell,



Such a cell is made up of five potentials e_1 , e_2 , e_3 , e_4 and e_5 . Potentials e_1 and e_5 are equal and oppositely directed, and the same is considered

⁴ Hoffman and Gortner, "Colloid Symposium Monograph II," Chemical Catalog Co., New York, 1925, p. 209. This work has been criticized by Cohn, *Physiol. Rev.*, 5, 349 (1925).

approximately true for e_2 and e_4 .⁵ Hence, the measured total potential of such a cell must be due almost entirely to the diffusion potential e_3 .

The potential measurements were made by means of a Queen Gray Standard type potentiometer which gave readings to 0.01 mv. The standard cell was a Weston cadmium cell which was checked against another cell certified by the Bureau of Standards. All measurements were carried out in an oil thermostat at 25° regulated to $\pm 0.015^\circ$. Observations were made by means of a telescope placed 12 feet from a Leeds and Northrup galvanometer No. 2500E with a sensitivity of 362 megohms.

The calomel-electrode vessels were of the Lewis type.⁶ The usual precautions were taken in making up the saturated potassium chloride electrodes and only the purest of chemicals used. The electrodes were checked with each other frequently and usually showed potentials below 0.10 mv. If at any time two reference electrodes showed potentials greater than 0.20 mv. they were discarded.

The hydrochloric acid solutions were made by dilution of a stock solution of 1 *N* hydrochloric acid which in turn was made from a standard, analyzed hydrochloric acid solution.

The concentration cells were made up and left in the bath for at least one hour before measurements were taken. When a cell had attained the temperature of the bath the stopcock was opened, the liquid junction established and a reading taken immediately. The stopcock was then closed. Another reading was taken shortly afterward. In all cases readings were taken with stopcock open; then the stopcock was immediately closed. Readings were taken in this manner at approximately hour intervals, until the potential of the cell started to drop rapidly. This usually resulted after a period of 40 hours.

The results obtained were very satisfactory. Three cells gave average values of 38.08, 38.08 and 38.11 mv. over a period of about 35 hours. These values represent the averages of about 20 readings taken over this period. The "time effects" were not large, amounting to less than 0.5 mv. It may be concluded, therefore, that the apparatus is capable of giving constant and reproducible potentials with this simple type of boundary.⁷

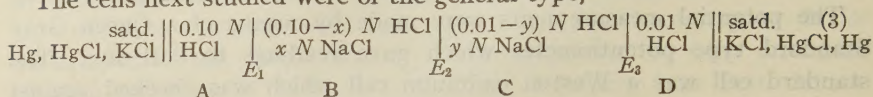
⁵ Loomis and Acree [*Am. Chem. J.*, **46**, 585 (1911)], Fales and Vosburgh [*This Journal*, **40**, 1291 (1918)] and others have shown that the diffusion potential between saturated potassium chloride and solutions of hydrochloric acid and sodium chloride below 0.1 *N* is zero or at any rate very small, that is, the order of a few tenths of a millivolt. More recently, Scatchard [*ibid.*, **47**, 696 (1925)] claims that the potential between saturated potassium chloride and concentrations of hydrochloric acid below 0.1 *N* is constant and has the value of 4.7 mv.

⁶ Lewis, Brighton and Sebastian, *ibid.*, **39**, 2245 (1917).

⁷ Calculated values of this boundary, by the Nernst equation, indicate that the potential closely approximates 38 mv.

Hydrochloric Acid-Sodium Chloride Cells

The cells next studied were of the general type,



The cells were made up of four solutions A, B, C and D. A represents 0.10 *N* hydrochloric acid, the same in all of the cells; B represents a mixture of hydrochloric acid and sodium chloride of total concentration 0.10 *N*, in which *x* varies in different cells from 0 to 0.10; C represents a mixture of hydrochloric acid and sodium chloride of total concentration 0.01 *N*, in which *y* is equal to *x* until the value 0.01 is reached and then remains constant while *x* in the other solution changes until it reaches its final value of 0.10; D represents a solution of 0.01 *N* hydrochloric acid, the same in all cells.

The sodium chloride solutions in the mixtures were formed by partial neutralization of the hydrochloric acid. The sodium hydroxide used for this purpose was made by dilution of the solution formed by the electrolysis of sodium amalgam. Its concentration was determined by titration with hydrochloric acid.

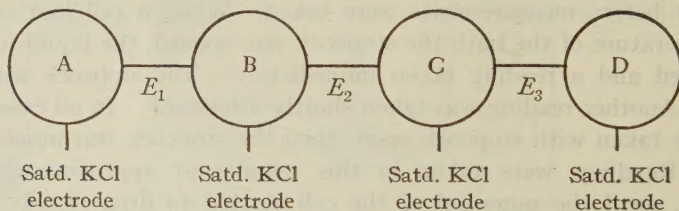


Fig. 2.—Arrangement of cell for diffusion potential measurements.

The total potential of the above cell is made up of three different diffusion potentials E_1 , E_2 and E_3 at the solution contacts A-B, B-C and C-D. The cell shown in Fig. 1 permits the measurement of a single diffusion potential. By means of three such cells joined together, and with the use of four saturated potassium chloride electrodes, it is possible to measure each of the three boundary potentials E_1 , E_2 and E_3 individually, or combinations of the three. The arrangement of the cell is illustrated by Fig. 2.²

The method of setting up the cells and the manner of taking the readings were exactly as described for the hydrochloric acid cells. After the cell had attained the temperature of the bath, readings were taken at hour intervals for a period of about 12 hours.

Table I serves as a typical example of the results obtained with Bound-

² It is apparent that each diffusion potential involves the measurement of perhaps three boundaries, but the two boundaries satd. KCl | solution and solution | satd. KCl, as noted before, probably have but little influence on the principal potential; hence, the diffusion potential is closely approximated in each case.

ary E_1 . This is characteristic of the results with Boundaries E_2 and E_3 . In the table the type cell is indicated at the top and the arrow from left to right indicates the direction of positive current through the cell. Duplicate cells were measured for each boundary and these are labeled Cell A and Cell B. The first entry under "Time" gives the hour at which the cell was first placed in the bath.

TABLE I
MEASUREMENTS WITH BOUNDARY E_1
Type Cell

Hg, HgCl, satd. KCl 0.10N HCl 0.08N NaCl satd. KCl, HgCl, Hg				0.02N HCl			
				$\xrightarrow{E_1}$			
Time	Cell A	Potential, mv.		Time	Cell B	Potential, mv.	
9:30 A. M.				9:45 A. M.			
11 A. M.		20.70		11 A. M.		21.11	
12 M.		21.02		12 M.		21.37	
1 P. M.		21.10		1 P. M.		21.34	
2 P. M.		21.06		2 P. M.		21.17	
3 P. M.		21.09		3 P. M.		21.03	
4 P. M.		21.00		4 P. M.		21.07	
5 P. M.		20.95		5 P. M.		20.88	
6 P. M.		21.03		7 P. M.		20.97	
7 P. M.		21.00		8 P. M.		20.90	
8 P. M.		21.06					
10 P. M.		21.02					
	Av.	+21.00			Av.	+21.08	

A summary of the measured values obtained with the various cells and the different boundaries is given in Table II. Col. 1 indicates the particular boundary measured; Col. 2, the value for the initial reading with Cell A; and Col. 3 the average of all readings for Cell A. Cols. 4 and 5 give similar readings with Cell B. Col. 6 gives the average of values in Cols. 3 and 5, and Col. 7, the averages of Cols. 2 and 4. The figures in parentheses after the potential values in Cols. 3 and 5 indicate the number of readings with the corresponding period in hours which the average value represents. For instance, (11-12) means 11 readings over a period of 12 hours.

An examination of the tables shows that the diffusion potentials of these more complex boundaries are as accurately reproducible as those of simpler systems, and in most instances appreciable "time effects" are not noticeable.

Hydrochloric Acid-Gelatin Cells

The hydrochloric acid-gelatin cells were of the following type,

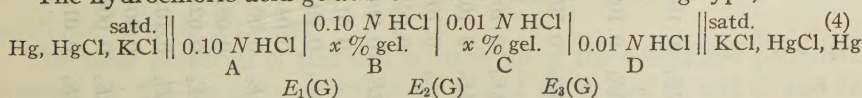


TABLE II
SUMMARY OF POTENTIALS FOR VARIOUS DIFFUSION BOUNDARIES

Boundary		Initial E , Cell A, mv.	Average total E , Cell A, mv.	(11-12)	+ 1.27	+ 1.09	(13-13)	Average total E , Cells A and B, mv.	Average initial E , Cells A and B, mv.
2E ₁	0.10N HCl	+ 0.79	+ 1.05	(11-12)	+ 1.27	+ 1.09	(13-13)	+ 1.07	+ 1.03
	0.004 NaCl								
3E ₁	0.10N HCl	2.12	2.18	(11-12)	1.96	1.91	(7-8)	2.04	2.04
	0.01N NaCl								
4E ₁	0.10N HCl	8.43	8.33	(12-12)	8.02	8.13	(10-12)	8.23	8.22
	0.04N NaCl								
5E ₁	0.10N HCl	20.70	21.00	(11-12)	21.11	21.08	(9-10)	21.04	20.90
	0.02N HCl								
6E ₁	0.10N HCl	30.30	29.94	(12-12)	30.37	29.99	(9-12)	29.93	30.33
	0.10N NaCl								
E ₂	0.10N HCl	38.52	38.08	(19-32)	38.57	38.08	(17-34)	38.08	38.54
	0.01N HCl								
2E ₂	0.004N NaCl	42.96	42.81	(11-12)	43.02	43.13	(11-12)	42.97	42.99
	0.006N HCl								
3E ₂	0.01N NaCl	55.71	55.54	(11-12)	56.14	56.09	(7-8)	55.81	55.92
	0.01N NaCl								
4E ₂	0.06N HCl	43.22	41.91	(12-12)	43.33	42.09	(11-12)	42.00	43.27
	0.01N NaCl								
5E ₂	0.08N NaCl	15.32	12.81	(11-12)	14.79	13.01	(9-10)	12.09	15.05
	0.02N HCl								
6E ₂	0.10N HCl	-10.70	-10.59	(12-12)	-10.51	-10.61	(9-11)	-10.60	-10.60
	0.01N NaCl								
2E ₃	0.004N NaCl	8.72	8.79	(11-12)	8.53	8.68	(11-12)	8.73	8.62
	0.006N HCl								
3E ₃	0.01N HCl	30.00	29.49	(12-11)	30.00	29.73	(11-11)	30.00	29.61
	0.01N NaCl								

These cells were made up of four solutions, A, B, C and D. A represents a solution of 0.10 *N* hydrochloric acid, the same in all cells. B represents a solution of $x\%$ gelatin in 0.10 *N* hydrochloric acid, in which x is the number of grams of gelatin present in 100 cc. of solution, and varies from 0 to 17.4 in the different cells. C represents a solution of $x\%$ gelatin

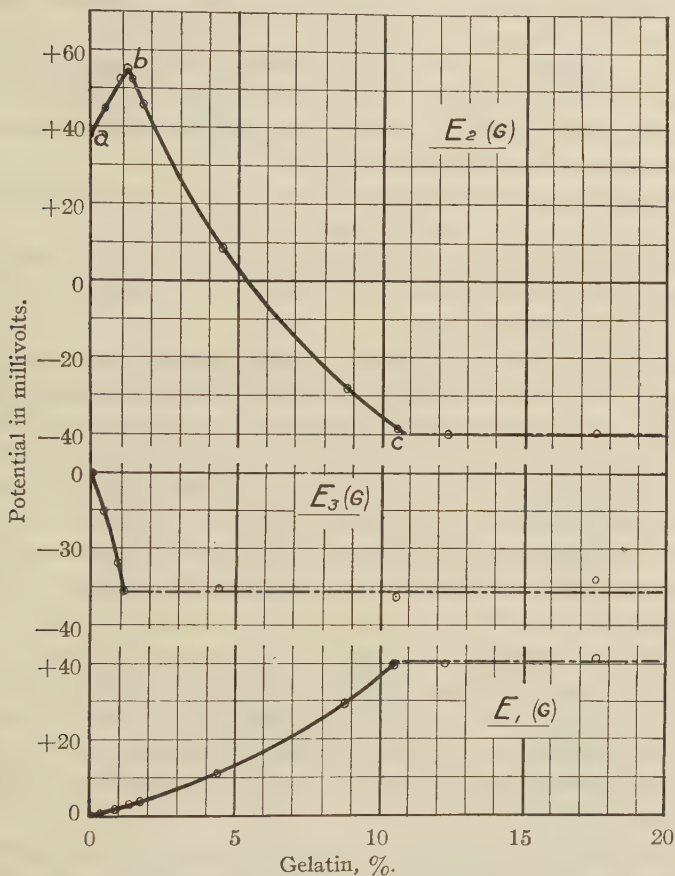


Fig. 3.—The diffusion potentials for boundaries $E_2(G)$, 0.10*N* HCl + $x\%$ gelatin | 0.01*N* HCl + $x\%$ gelatin; $E_3(G)$, 0.10*N* HCl + $x\%$ gelatin | 0.01*N* HCl; $E_1(G)$, 0.10*N* HCl | 0.10*N* HCl + $x\%$ gelatin.

in 0.01 *N* hydrochloric acid, where x again represents the number of grams of gelatin in 100 cc. of solution and varies between the limits 0 and 17.4 in the different cells. D represents a solution of 0.01 *N* hydrochloric acid, the same in all cells.

The total potential of this cell is made up of three diffusion potentials formed by the contacts of Solutions A and B, B and C, and C and D.

These are E_1 (G), E_2 (G) and E_3 (G) as indicated. Four saturated potassium chloride electrodes, one dipping in each solution, permit the measurement of any boundary or combination of boundaries.⁹

The hydrochloric acid solutions were made by dilution of an analyzed solution of 1 *N* hydrochloric acid, as described with the previous cells.

The gelatin was a pure ash-free (0.04% ash) iso-electric gelatin obtained from the Research Laboratories of the Eastman Kodak Company. The moisture content of the gelatin was about 12.6%. This was determined by heating 2 to 3g. samples in the drying oven at 110° until constant weight was obtained. The moisture content was applied as a correction to all weighings of gelatin.

A uniform procedure was used in preparing the gelatin solutions. The manner in which a 2% solution (which corresponds to a 1.75% solution when the moisture correction is applied) of gelatin in 0.10 *N* hydrochloric acid was prepared serves as an illustration of the general way in which all solutions were obtained.

Ten g. of gelatin (8.74 g. of the dry gelatin) was weighed to the nearest milligram. This was dissolved in distilled water at a temperature of about 35°. The solution of gelatin was then poured into a 500cc. flask and water added so that the volume was between 300 and 400 cc. A volume of 50 cc. of 1 *N* hydrochloric acid was then run in by means of a calibrated pipet and water was added to bring the volume to 500 cc. The solution of 2% gelatin in 0.01 *N* hydrochloric acid was made up in exactly the same way, 50 cc. of the 0.10 *N* hydrochloric acid being used instead of 1 *N* hydrochloric acid.

There was no difficulty encountered in making up these solutions, although with higher concentrations of gelatin they were very viscous and set to gels within a short time. The gel was easily melted by placing the flask in warm water. After this procedure it would not set to a gel until some time afterward, so that there was no trouble in filling the cells and making the boundaries.

The cells were filled and the boundaries made in exactly the same manner as previously described with the hydrochloric acid-sodium chloride cells. Readings were extended over a much longer period than with the other systems, usually over 24 hours. Since it seemed desirable to experiment with these cells under as rigorous conditions as possible, so that any consistent or reproducible results which might be obtained could not necessarily be ascribed to an empirical procedure, duplicate cells were measured under as varied conditions as possible, and in many instances entirely new gelatin solutions were made up. All measurements were carried out at 25°.

⁹ With considerations as noted before (see Ref. 5).

Results

The information given in Table III is typical of the results obtained with other cells. This table gives measurements with cells containing 0.874% of gelatin.

TABLE III
MEASUREMENTS ON CELLS WITH 0.874% OF GELATIN
Type Cell

$\text{Hg, HgCl, KCl} \parallel \text{satd.} \parallel \begin{array}{c} 0.10 \text{ } N \\ \text{HCl} \end{array} \parallel \begin{array}{c} 0.10 \text{ } N \\ 0.874\% \text{ gel.} \end{array} \parallel \begin{array}{c} 0.01 \text{ } N \\ 0.874\% \text{ gel.} \end{array} \parallel \begin{array}{c} \text{HCl} \\ \text{HCl} \end{array} \parallel \text{satd.} \parallel \text{KCl, HgCl, Hg}$					
$\begin{array}{ccccc} E_1(G) & & E_2(G) & & E_3(G) \\ \xrightarrow{\hspace{1cm}} & & \xrightarrow{\hspace{1cm}} & & \xleftarrow{\hspace{1cm}} \\ & \xrightarrow{\hspace{3cm}} & & & \end{array}$					
Cell A					
Time	$E_1(G)$, mv.	$E_2(G)$, mv.	$E_3(G)$, mv.	$E(G)$, mv.	$E_s(G)$, mv.
5:30 P. M.					
7 P. M.	1.95	52.32	24.57	29.30	29.70
7:45 P. M.	1.87	52.32	23.57	30.70	30.62
8:30 P. M.	2.19	52.45	23.05	30.86	31.59
9:15 P. M.	1.86	52.31	23.03	31.03	31.14
10:15 P. M.	1.85	52.30	23.06	31.13	31.09
11:10 P. M.	1.87	52.34	23.06	31.16	31.15
9:15 A. M.	2.20	52.01	23.72	30.13	30.49
10:30 A. M.	1.95	52.10	23.72	30.41	30.33
11:45 A. M.	2.03	52.26	23.55	30.62	30.74
6:30 P. M.	1.86	52.35	23.48	30.76	30.73
8 P. M.	1.88	52.30	23.55	30.53	30.63
10 P. M.	2.14	52.03	23.45	30.58	30.72
9:30 A. M.	1.86	52.04	23.24	30.59	30.74
Av.	+1.96	+52.24	-23.46	+30.59	+30.74
Cell B					
10:30 A. M.					
12 M.	1.95	52.11	25.28	28.75	28.98
1:30 P. M.	2.07	52.02	24.83	29.26	29.26
2:30 P. M.	1.88	52.28	23.65	30.22	30.51
4 P. M.	1.93	52.25	23.84	30.41	30.34
5:15 P. M.	2.08	52.23	24.33	30.02	29.48
7 P. M.	1.90	52.45	23.97	30.32	30.41
8 P. M.	1.84	52.52	24.01	30.35	30.44
9 P. M.	1.82	52.28	24.05	30.28	30.05
10 P. M.	2.08	52.17	24.22	30.04	30.03
9 A. M.	1.77	52.05	23.82	29.82	30.00
12:30 P. M.	1.88	52.11	23.75	29.86	30.04
9:30 P. M.	1.85	51.97	23.72	29.93	30.10
Av.	+1.90	+52.20	-24.11	+29.95	+29.97

At the top of the table is indicated the type cell with the boundaries $E_1(G)$, $E_2(G)$ and $E_3(G)$. Small arrows give the direction of flow of positive current in each boundary and a large arrow indicates the positive

or negative character of the entire cell; if from left to right the potential is considered to be positive and if from right to left negative. The first half of the table gives the measured values with Cell A and the second half those with Cell B. The first entry under "Time" gives the hour at which the cell was first placed in the thermostat bath. Successive entries give the time when measurements on the various boundaries were made. The readings in millivolts obtained with the three boundaries are given in the columns labeled $E_1(G)$, $E_2(G)$ and $E_3(G)$. In columns $E(G)$ and $E_s(G)$ are given the measured values for the total cell and also the values as determined from the algebraic sums of the boundaries.

It can be seen in both Cells A and B that in most instances the "time changes" in boundaries are very small, being of the order of a few tenths of a millivolt. The averages of the duplicate cells are very good.

Cells containing 0.437, 1.31, 1.75, 4.47, 8.74, 10.49, 12.23 and 17.48% of gelatin were treated in a manner similar to that illustrated in Table III.¹⁰

With higher concentrations of gelatin the "time changes" in boundaries $E_1(G)$ and $E_2(G)$ became more noticeable, although they consisted of fluctuations from hour to hour and did not show a general drift in any given direction. The 8.74% gelatin solutions became very viscous and overnight the gelatin in 0.01 *N* hydrochloric acid set to a gel. This had little effect on the boundary as comparison between readings taken at night and those taken the following morning showed only a change of 0.35 mv. and 0.20 mv. on two separate cells. With the 17.48% gelatin cells both gelatin solutions changed to gels within a few hours, but this change in state showed no noticeable effect on any of the potentials. In general, the agreement between duplicate cells and the deviations in readings in the single boundaries were not so good with the higher concentrations of gelatin, that is, above 10% of gelatin, but the potentials were perfectly definite. However, this irregularity at the higher concentrations introduced no difficulty since the significant conclusions are based upon results obtained at lower concentrations.

In practically every case values for duplicate cells agreed to less than 0.5 mv. and in most cases to within a few tenths of a millivolt. This shows that diffusion potentials with the supposedly complex gelatin systems are as accurately reproducible and as definite as with the simpler hydrochloric acid-sodium chloride systems.

In the cells containing more than 1.09% of gelatin, Boundary $E_3(G)$ showed a rather erratic behavior, the initial value for the boundary being much larger than any of the other values. This fall in potential amounted to as much as 10.7 mv. in ten hours. The reason for the apparent "time

¹⁰ The original thesis may be consulted for detailed measurements on these cells.

effect" in this boundary and others was made the subject of a special investigation and has been satisfactorily explained.

The graphical representation of the changes in diffusion potential in the different cells with the amount of gelatin added is given in Fig. 3, Curves $E_1(G)$, $E_2(G)$ and $E_3(G)$. In all instances the percentage of gelatin added to the solutions is plotted against the potential in millivolts.

Curve $E_1(G)$ shows the change in potential for Boundary $E_1(G)$ with increasing amounts of gelatin where x , the amount of gelatin in 100 cc. of solution, varies from 0.437 to 17.4 g. This potential starts at zero, since there is 0.10 *N* hydrochloric acid on each side of the boundary, increases gradually as gelatin is added to one side and finally reaches a maximum of +40 mv., when the gelatin concentration is about 10.9%. Beyond this concentration, as more gelatin is added, the potential remains practically constant in the region of +40 mv.

Curve $E_3(G)$ shows the changes in potential for Boundary $E_3(G)$. The potential starts at zero, since 0.01 *N* hydrochloric acid is on both sides of the boundary. As gelatin is added to one of these solutions, that is, as x varies from 0.437 to 17.4, the potential becomes increasingly more negative very rapidly, and soon reaches a limiting value of -31.50 mv. at 1.09% gelatin concentration. With higher concentrations of gelatin the potentials become largely indeterminate, although they tend to remain in the region of -31 mv.

Curve $E_2(G)$ represents Boundary $E_2(G)$, where x again varies from 0.437 to 17.4. The potential for this boundary starts with that between 0.10 *N* and 0.01 *N* hydrochloric acid, increases gradually as equal amounts of gelatin are added to both solutions and finally reaches a maximum of +54.97 mv. at a concentration of 1.09%. As more gelatin is added to both sides, that is, as x continues to increase, the potential drops rapidly, becomes negative and finally reaches a minimum of about -39 mv. It then remains constant for higher gelatin concentrations. The minimum point on the curve corresponds to a gelatin concentration of about 10.9%.

In Fig. 4 are given graphically the results with the hydrochloric acid-sodium chloride cells which have been previously described. The boundaries are indicated under each curve. The number of cc. of sodium hydroxide solution added to produce the hydrochloric acid-sodium chloride mixtures are plotted against the resulting potentials in millivolts.

Equivalent Weight of Gelatin from Diffusion-Potential Curves

If the curves for the hydrochloric acid-sodium chloride systems are compared with the curves for the hydrochloric acid-gelatin systems, it will be noted that their general appearance is strikingly similar. This would seem to indicate that the mechanism which is the cause of the

potentials in the hydrochloric acid-gelatin systems is perhaps similar to that of the hydrochloric acid-sodium chloride systems and interpretations that might be applied to the latter could also be applied to the former.

In the hydrochloric acid-sodium chloride curves, the maximum and minimum points on each curve correspond to the condition where complete

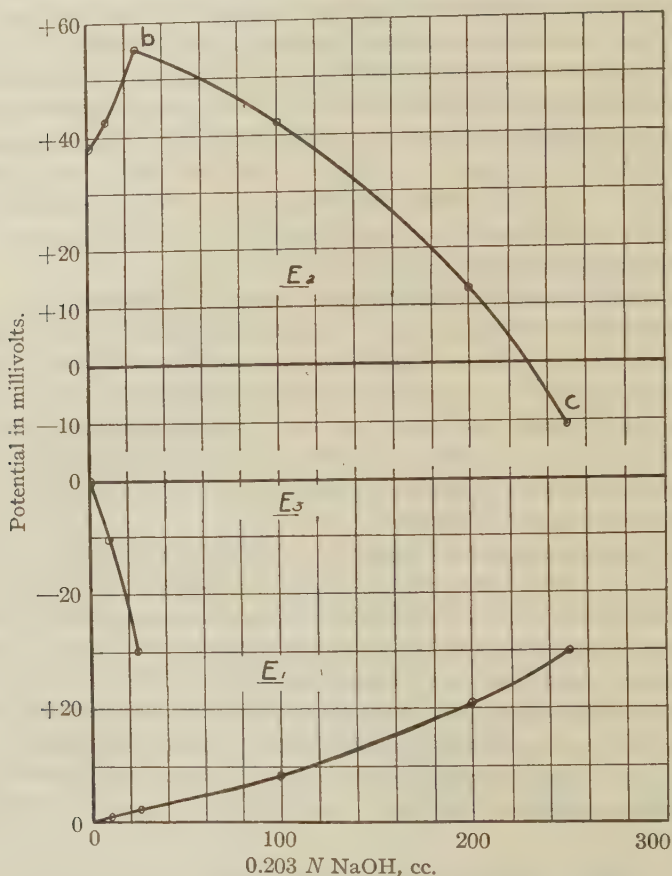


Fig. 4.—Diffusion potential changes with boundaries E_2 $(0.10 - x) N HCl + x N NaCl | (0.1 - y) N HCl + y N NaCl$; E_3 $(0.01 - y) N HCl + y N NaCl | 0.010 N HCl$; E_1 $0.10 N HCl | (0.10 - x) N HCl + x N NaCl$.

combination between hydrochloric acid and sodium hydroxide has occurred. It seems clear from examination of the hydrochloric acid-gelatin curves that the four points of inflection on these three curves must indicate definite regions where complete combination between gelatin and hydrochloric acid has taken place. On both Curves $E_2(G)$ and $E_3(G)$ the first breaks occur at a gelatin concentration of 1.09%. This would correspond

to an equivalent weight of gelatin of 1090, since the solution at this point contains 1.09 g. of gelatin in 100 cc. of 0.10 *N* hydrochloric acid, and hence 1090 g. of gelatin would be necessary to combine with one equivalent weight of hydrochloric acid. On Curve $E_1(G)$ the first break and on Curve $E_2(G)$ the second break occur at a concentration of close to 10.9%, which corresponds to a condition where gelatin has combined with all the 0.10 *N* hydrochloric acid. This again gives the equivalent weight of gelatin as close to 1090.

These curves offer a striking proof that combination between gelatin and hydrochloric acid at concentrations ranging from 0.01 *N* to 0.10 *N* occurs in stoichiometric proportions, and the amount of gelatin which will combine with one equivalent weight of hydrochloric acid is 1090.

Other values which have been obtained for the equivalent weight of gelatin are given in a summarized form in Table IV. The results obtained in this work compare favorably with Hitchcock's values and also with those calculated by Greenberg and Schmidt.

TABLE IV

VALUES FOR THE EQUIVALENT WEIGHT OF GELATIN

Equiv. wt	Method	Equiv. wt.	Method
665	Electrometric titration ^a	1319-2083	Titration ^h
839	Titration ^b	1180	Titration ⁱ
1428	Titration ^c	1087	Electrometric titration ^j
1176	Calcd. ^c	1120	Corr. of above and from diamnized gelatin ^k
1250	Estimated ^d	1160	Conductivity ^l
1063	Electrometric titration ^e	1135	Calcd. ^m
839	Catalytic action ^f	1180	Electrometric titration ⁿ
885	Electrometric titration ^g		

^a Manabe and Matula, *Biochem. Z.*, **52**, 369 (1913).

^b Procter, Ref. 2c.

^c Bracewell, Ref. 3d.

^d Lloyd, *Biochem. J.*, **14**, 147 (1920).

^e Lloyd and Mayes, *Proc. Roy. Soc.*, **B93**, 69 (1922).

^f Wintgren and Kruger, *Kolloid-Z.*, **28**, 81 (1921).

^g Wintgren and Vogel, *ibid.*, **30**, 45 (1922).

^h Oakes and Davis, *J. Ind. Eng. Chem.*, **14**, 706 (1922).

ⁱ Loeb, Ref. 2a.

^j Hitchcock, *J. Gen. Physiol.*, **4**, 733 (1922).

^k Hitchcock, *ibid.*, **6**, 95 (1923).

^l Ref. *k*, p. 201.

^m Greenberg and Schmidt, *Proc. Soc. Exptl. Biol. Med.*, **21**, 281 (1924).

ⁿ Atkin and Douglas, *J. Am. Leather Chem. Assocn.*, **19**, 528 (1924).

Summary

1. A cell system is described which allows a close approximate measurement of diffusion potentials.

2. Reproducible diffusion potentials which show but small "time effects" have been measured with hydrochloric acid systems, hydrochloric acid-sodium chloride systems and hydrochloric acid-gelatin systems.

3. The reaction between hydrochloric acid and gelatin appears to be stoichiometric, purely chemical in nature.

4. A new and unusual method for determining the combining capacity of gelatin for hydrochloric acid is given. Four independent boundary measurements give the equivalent weight of gelatin as close to 1090.

Work of this nature is to be continued with di- and tri-basic acids and bases with gelatin, also for similar systems in which gelatin will be replaced by other proteins.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN]
**DIFFUSION-POTENTIAL MEASUREMENTS APPLIED TO
HYDROCHLORIC ACID-GELATIN SYSTEMS. II. THE
COMPONENTS OF HYDROCHLORIC ACID-GELATIN
SOLUTIONS¹**

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A previous article² has shown that the equivalent weight of gelatin can be obtained from diffusion-potential measurements on hydrochloric acid-gelatin systems and that the reaction between hydrochloric acid and gelatin appears to be a stoichiometric one. This article explains the irregularities that were shown in some of the previous measurements³ and gives a more complete understanding of the conditions as existing in the various boundaries and solutions. All references to curves of Figs. 3 and 4 in this article refer to the same figures of the previous article.

The Effect of Uncombined Gelatin at the Diffusion Boundary

From comparison of curves in Fig. 3 with those in Fig. 4 it will be seen that the hydrochloric acid-gelatin solutions were not exactly similar to the hydrochloric acid-sodium chloride mixtures, as gelatin was added

¹ The material presented in this article is constructed from a portion of the thesis submitted to the Graduate School of the University of Michigan by Egbert K. Bacon, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² Ferguson and Bacon, *THIS JOURNAL*, **49**, 1921 (1927).

³ Portions of Curve $E_2(G)$ were concave upward, while similar portions of Curve E_2 were concave downward. Large "time effects" were observed in certain regions of curves $E_1(G)$, $E_2(G)$ and $E_3(G)$.

to the hydrochloric acid without regard to the amount needed for complete combination. (In fact, this amount was not known until after the experiments were carried out and the equivalent weight was determined.) Thus it happened that in many cases there was uncombined gelatin present in the acid-gelatin mixtures.

With the hydrochloric acid-sodium chloride mixtures free sodium hydroxide was never present. If sodium hydroxide had been added to the 0.01 *N* hydrochloric acid after complete neutralization had occurred (which corresponds to the regions beyond the maximum point *b* on Curve E_2), the condition at the boundary would have been

$$(0.10 - x) \text{ } N \text{ HCl, } x \text{ } N \text{ NaCl} \mid 0.01 \text{ } N \text{ NaCl, } y \text{ } N \text{ NaOH} \quad (1)$$

At such a diffusion boundary there is a new source of potential, that between *x* *N* hydrochloric acid and *y* *N* sodium hydroxide which, according to Planck,⁴ would be largely indeterminate because chemical action between sodium hydroxide and hydrochloric acid takes place at the boundary.

This appears to be similar to the condition which resulted in the *b c* region of Curve $E_2(G)$. The point *b* on the curve corresponds to a condition of complete combination between gelatin and 0.01 *N* hydrochloric acid so that instead of writing the boundary as

$$0.10 \text{ } N \text{ HCl, } x\% \text{ gel.} \mid 0.01 \text{ } N \text{ HCl, } x\% \text{ gel.} \quad (2)$$

a more exact condition is given as

$$(0.10 - x) \text{ } N \text{ HCl, } y\% \text{ combined gel.} \mid 0.01 \text{ } N \text{ combined gel., } z\% \text{ uncombined gel.} \quad (3)$$

Thus, there was free hydrochloric acid and uncombined gelatin in contact at the boundary, until complete combination between the 0.10 *N* hydrochloric acid and gelatin had occurred, that is, a concentration of 10.9% of gelatin.

"Corrected" Curve for Boundary $E_2(G)$

If the above is the explanation of the real conditions existing at the contact boundary in the region *b c* of Curve $E_2(G)$, then a boundary such as

$$(0.10 - x) \text{ } N \text{ HCl, } y\% \text{ combined gel.} \mid 0.01 \text{ } N \text{ combined gel.} \quad (4)$$

should correspond more nearly to the condition of the same region on curve E_2 of the hydrochloric acid-sodium chloride system,

$$(0.10 - x) \text{ } N \text{ HCl, } x \text{ } N \text{ NaCl} \mid 0.01 \text{ } N \text{ NaCl} \quad (5)$$

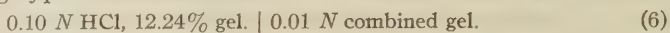
Accordingly, boundaries such as that given in (4) were measured. The results of these "corrected" measurements are given in Fig. 1 A, corresponding to 1.75, 4.37, 8.74 and 10.49% gelatin.

The path of the curve *b'c'* between 1.09% of gelatin and 10.9% of gelatin is now concave downward, the same as in Curve E_2 . It converges to the same point *c'*, as does Curve $E_2(G)$, when complete combination

⁴ Planck, *Wied. Ann.*, **40**, 561 (1890).

between 0.10 *N* hydrochloric acid and gelatin has taken place (10.9% of gelatin). This is further proof that the equivalent weight of gelatin is close to 1090, as shown in the previous article.

To show that uncombined gelatin present in the left-hand side of the boundary would have no appreciable effect on the potential after all of the free hydrochloric acid had combined with the gelatin, a boundary of the following type was measured.



This should give a potential similar to a boundary such as



since there is no free hydrochloric acid present in either system, but only combined and uncombined gelatin.

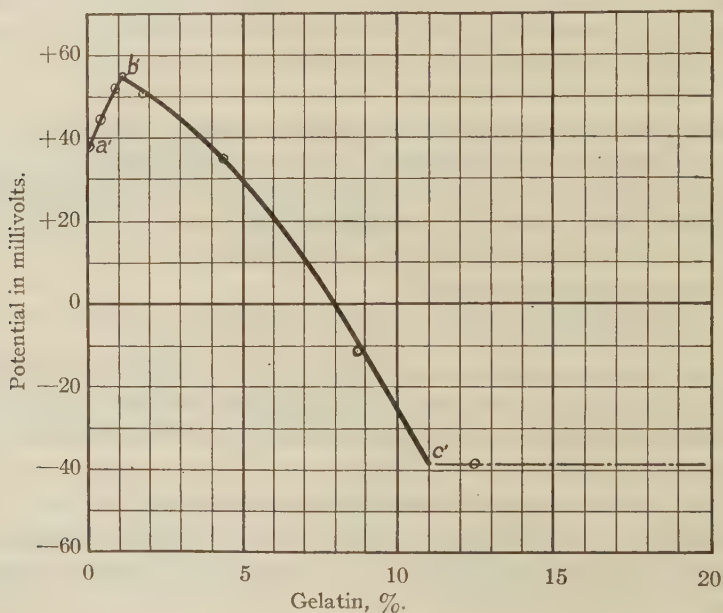


Fig. 1A.— $E_2(G)$, 0.01 *N* HCl + *x*% gelatin $\mid$ 0.01 *N* HCl + *y*% gelatin, where *x* varies from 0 to 10.9 and *y* varies from 0 to 1.09.

The potentials of these two boundaries were -37.69 mv. and -39.79 mv., respectively. This close agreement shows that an excess of gelatin beyond the amount required for complete combination with the acid present has no appreciable influence on the diffusion potential.

The Function of Free Hydrochloric Acid and Uncombined Gelatin at the Diffusion Boundary

From these considerations it seems clear that the difference in observed potentials, as shown by Curves $E_2(G)$ and $2E_2(G)$, between points *b*

and c and b' and c' is a function of the concentration of free hydrochloric acid on one side of the boundary and the concentration of the uncombined gelatin on the other. It is the presence of these components on opposite sides of the contact layer that is the cause of the differences in diffusion potentials.

This is illustrated more conclusively by the following considerations. Any point on the $b'c'$ region of Curve $2E_2(G)$ represents the boundary condition as

$$(0.10 - x) N \text{ HCl, } y \% \text{ combined gel. } | 0.01 N \text{ combined gel.} \quad (8)$$

If z per cent. of iso-electric gelatin were added to the $0.01 N$ combined gelatin, any change resulting in the diffusion potential would be caused by the effect produced by the introduction of this excess of iso-electric gelatin. This is exactly the conditions that are represented in the region $b'c$ of Curve $E_2(G)$, only it gives the results of proportionally increasing amounts of uncombined gelatin in contact with decreasing amounts of hydrochloric acid. If one of these factors is kept constant, the effect produced by changing quantities of the other can be observed. A boundary of the type

$$0.10 N \text{ HCl, } 1.75\% \text{ gel. } | 0.01 N \text{ combined gel., } x\% \text{ uncombined gel.} \quad (9)$$

illustrates such a condition. Boundaries of this nature were measured.

The solution in the left-hand side contained 1.75% of gelatin in $0.10 N$ hydrochloric acid or $0.084 N$ hydrochloric acid plus combined gelatin. The composition of this solution was kept constant. Varying quantities of iso-electric gelatin (which remained in the uncombined state) were added to the combined gelatin solution on the other side of the boundary.

The results of these measurements are given graphically in Fig. 2A, Curve 2(G)A. The potential is plotted as a function of the value of x , and the values of x used are 0, 0.66, 1.53, 4.15, 5.03 and 11.25. The curve appears to reach a limiting value at a potential around +15 mv. Concentrations of gelatin beyond 5 or 6% produce little effect.

Curve 2(G)B represents a boundary of the type

$$0.10 N \text{ HCl, } 5.24\% \text{ gel. } | 0.01 N \text{ combined gel., } x\% \text{ uncombined gel.} \quad (10)$$

This is similar to Boundary 9 but represents a lower starting point on $b'c'$, that is, where the free hydrochloric acid on the left-hand side of the boundary has the lower concentration, 0.052. The values for x are 0, 4.15 and 5.9. Concentrations of gelatin beyond 4% show little effect on this boundary.

Curve 2(G)C shows the results when the free acid has the still lower concentration, $0.0084 N$. Constancy in potential is reached between 1 and 2% of gelatin.

Curve 2(G)D shows a condition of constancy when there is no free hydrochloric acid left. Any concentration of gelatin now has no appreciable effect on the potential.

To show further the difference between the effect of combined and uncombined gelatin the following boundary was measured,

$$0.10 \text{ N HCl, } 1.74\% \text{ gel.} \mid 4.37\% \text{ solution combined gel.} \quad (11)$$

in which one solution represents 1.75% of gelatin in 0.10 N hydrochloric acid and the other a 4.37% solution of *combined* gelatin, based upon the combining weight 1090 for gelatin. The value -39.19 mv. was obtained for the potential of this boundary. A similar boundary which contained 4.37% of *iso-electric* gelatin in 0.01 N hydrochloric acid gave a value of -8.61 mv.

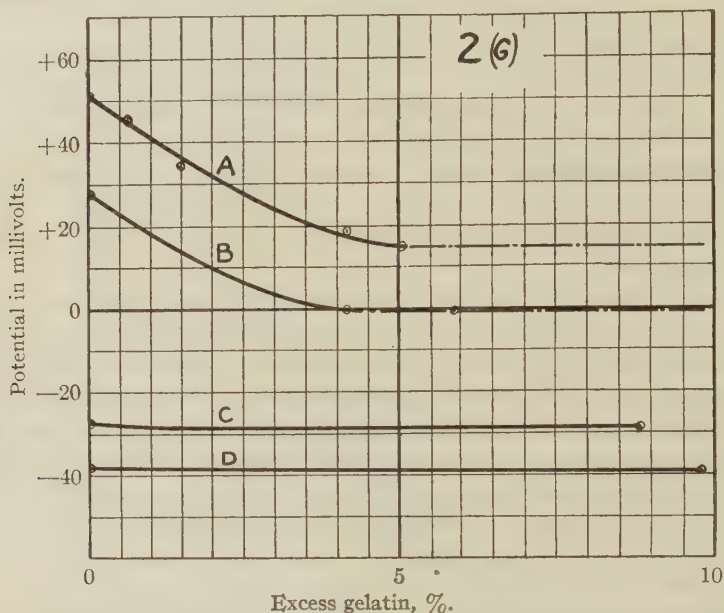


Fig. 2A.—The influence on diffusion potentials when uncombined gelatin is on one side of the boundary and free HCl on the other. Curve A, 1.7% gelatin, 0.084 N HCl; B, 5.2% gelatin, 0.052 N HCl; C, 10% gelatin, 0.008 N HCl; D, 10.9% gelatin, 0.000 N HCl.

Conclusions

The importance of defining hydrochloric acid-gelatin solutions in terms of free acid, combined and uncombined gelatin, is shown in these experiments. This can be done only if the stoichiometric character of the gelatin-acid combination is admitted and the equivalent weight of gelatin known. The understanding of the conditions existing in these hydrochloric acid-gelatin solutions and the cause of the observed potentials become clear when the following assumptions are made.⁵

- (1) Iso-electric gelatin combines stoichiometrically with hydrochloric

⁵ These are essentially the same as those of Loeb and his co-workers.

acid, at least between concentrations of 0.10 *N* and 0.01 *N*. Other interpretations of the curves which have been discussed could hardly lead to other conclusions. Five independent boundary measurements establish the equivalent weight of gelatin as close to 1090.

(2) Iso-electric gelatin, although considered neutral, is able by its amphoteric character to combine with hydrochloric acid much in the same manner as hydrochloric acid combines with sodium hydroxide. A highly ionized salt, gelatin chloride, is formed which is dissociated into a gelatin cation and a chloride anion.

From a consideration of these assumptions the diffusion potentials of hydrochloric acid-gelatin systems become understandable. When the components of the solutions are gelatin chloride (combined gelatin) and free hydrochloric acid, the observed potentials are very much the same as if the components were hydrochloric acid and sodium chloride. The introduction of any uncombined gelatin at one of the diffusion boundaries where free hydrochloric acid is present results in interaction between hydrochloric acid and gelatin at the boundary layer, with the formation of gelatin chloride. Thus the diffusion layer would be largely indeterminate and the potential would have no specific value or precise meaning. Examination of the experimental data and curves prove this, as erratic results were obtained whenever free hydrochloric acid and uncombined gelatin were at the boundary. This is in accord with Planck's views that a diffusion potential which results at a boundary where there is chemical action is largely indeterminate.

Summary

Important conclusions resulting from this investigation may be summarized as follows:

1. The components of hydrochloric acid-gelatin solutions should be defined in terms of free acid, combined and uncombined gelatin.
2. Diffusion potentials of hydrochloric acid-gelatin systems are only understandable if the hydrogen chloride-gelatin combination results in the formation of highly-ionized gelatin chloride.

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